

Supplementary Figures

as part of the manuscript

***Bordetella pertussis* outer membrane vesicle vaccine confers equal efficacy in mice with milder inflammatory responses compared to a whole-cell vaccine**

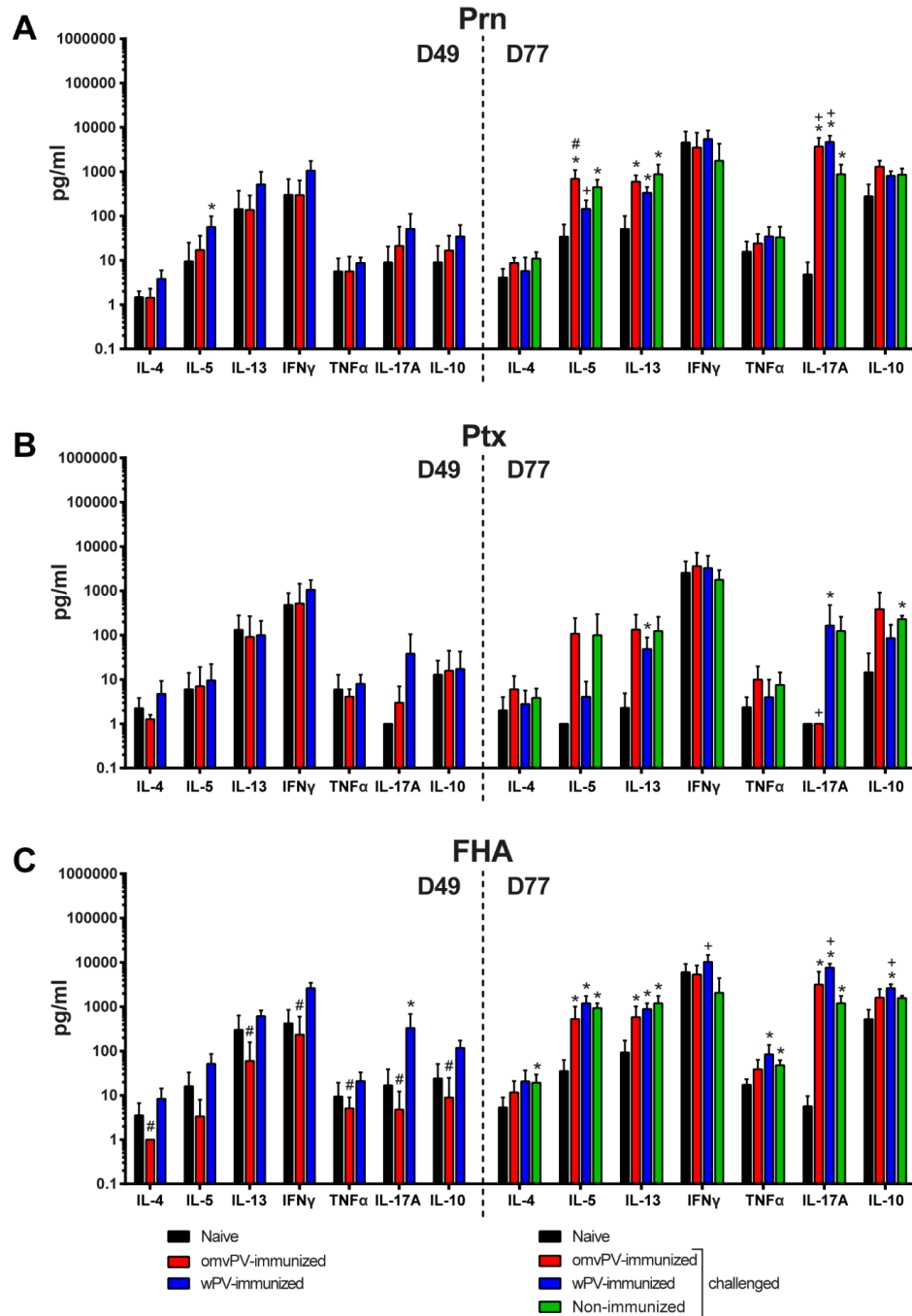
René H. M. Raeven^{1,2*}, Jolanda Brummelman³, Jeroen L. A. Pennings⁴, Larissa van der Maas¹, Wichard Tilstra¹, Kina Helm³, Elly van Riet¹, Wim Jiskoot², Cécile A. C. M. van Els³, Wanda G. H. Han³, Gideon F. A. Kersten^{1,2}, Bernard Metz¹

¹ Institute for Translational Vaccinology (Intravacc), Bilthoven, The Netherlands,

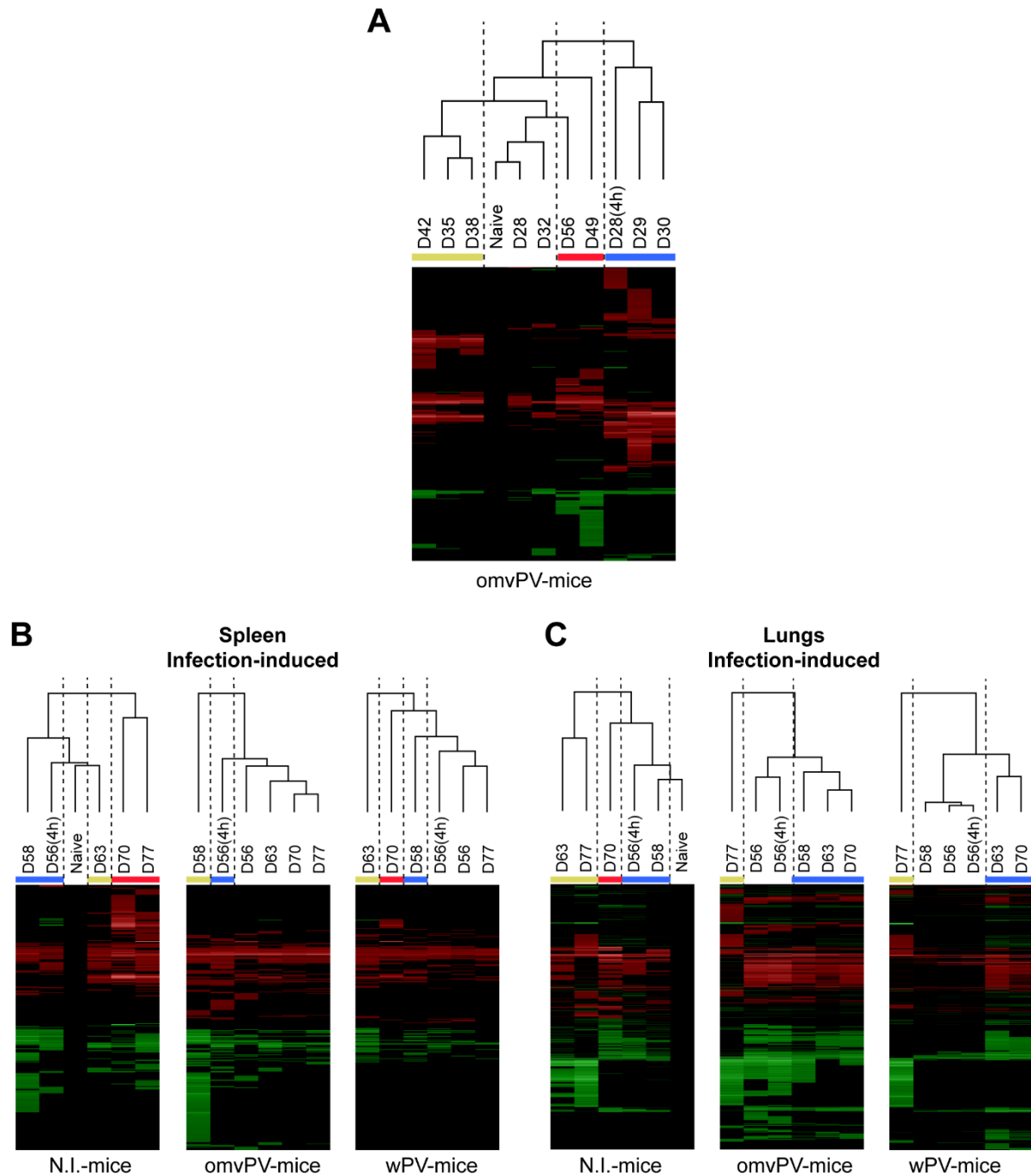
² Division of Drug Delivery Technology, Leiden Academic Centre for Drug Research, Leiden, The Netherlands,

³ Centre for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands,

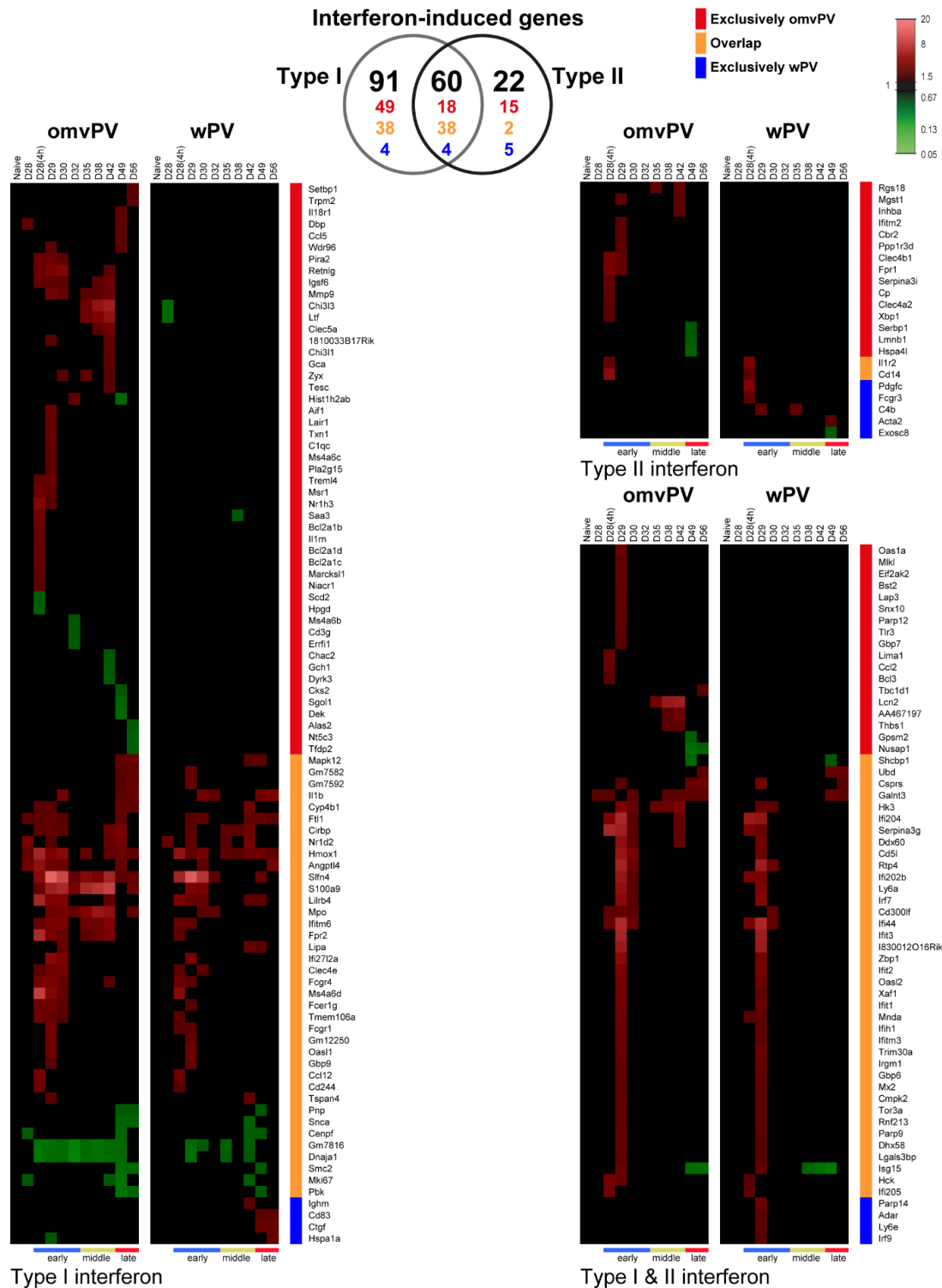
⁴ Centre for Health Protection (GZB), National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands



Supplementary Figure S1. Splenic cytokine responses after antigen restimulation in omvPV- and wPV-immunized mice as compared to naive control mice before and after challenge. (A-C) Concentrations of IL-4, IL-5, IL-13, IFN γ , TNF α , IL-17A and IL-10 were determined in the culture supernatants after 7 day stimulation of splenocytes with 1 μ g/ml Prn, Ptx, or FHA. Splenocytes were harvested post booster immunization (day 49, left panel) of mice immunized with omvPV (red) or wPV (blue). Post-challenge (day 77, right panel), same groups were included with an additional group of non-immunized mice that received a challenge (green). In both experiments, complete naive mice (black) were used as control. Results for each mouse are corrected for medium stimulation. * = $p \leq 0.05$ for immunized group and challenged group vs. naive group, # = $p \leq 0.05$ for omvPV group vs. wPV group, + = $p \leq 0.05$ for challenged immunized group vs. challenge non-immunized group.

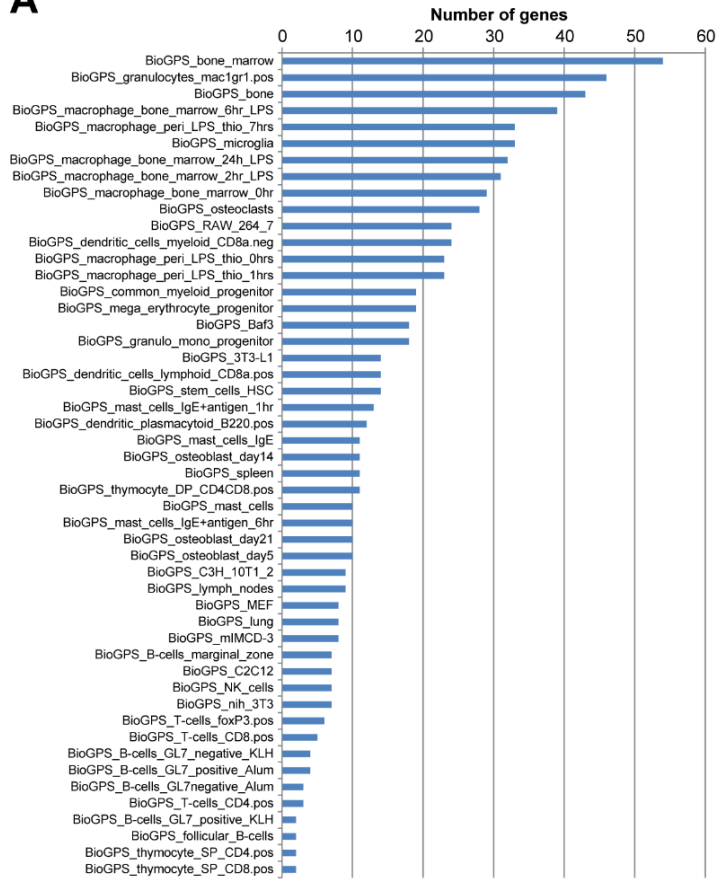


Supplementary Figure S2. Hierarchical clustering. (A) Hierarchical clustering of the omvPV-induced splenic transcriptome dataset to identify which time points showed a similar response. The response was divided in four parts illustrated by the different colors. (B-C) Hierarchical clustering on the (B) splenic and (C) pulmonary transcriptome datasets of challenged N.I.-mice, omvPV-mice and wPV-mice to identify which time points showed a similar response.

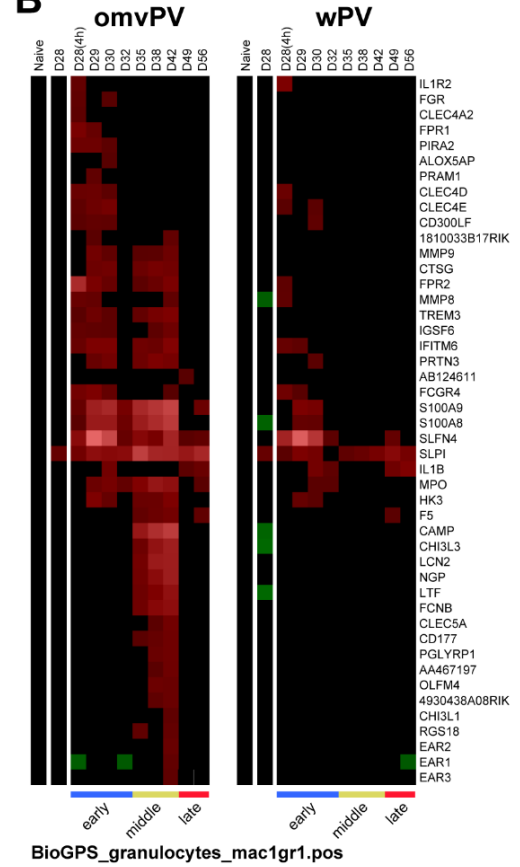


Supplementary Figure S3. IFN-induced genes in spleens of omvPV- and wPV-immunized mice. Genes induced by omvPV and wPV in the transcriptome of the spleen were matched with the Interferome database (<http://www.interferome.org/interferome/home.jspx>). A Venn diagram shows the total number of genes (black) induced by type I IFN, type II IFN, or both. Genes that were found exclusively in the omvPV-mice (red), wPV-mice (blue) or overlapped in both groups (orange) are depicted. The individual genes involved in type I IFN, type II IFN, or both are shown in heatmaps with the corresponding color codes of immunization background.

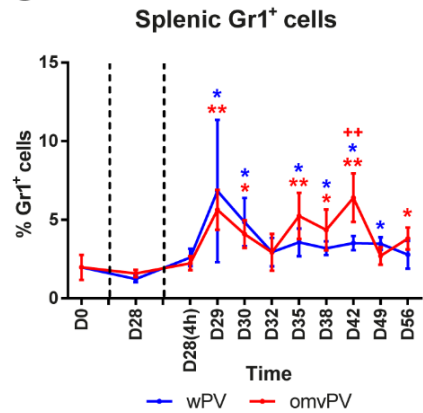
A



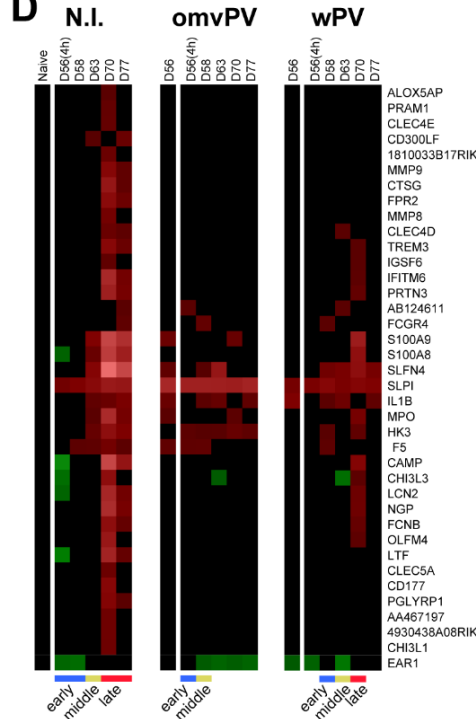
B



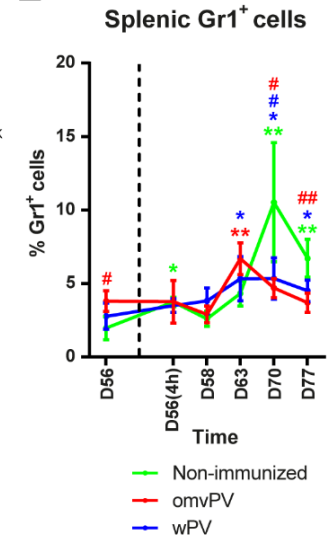
C



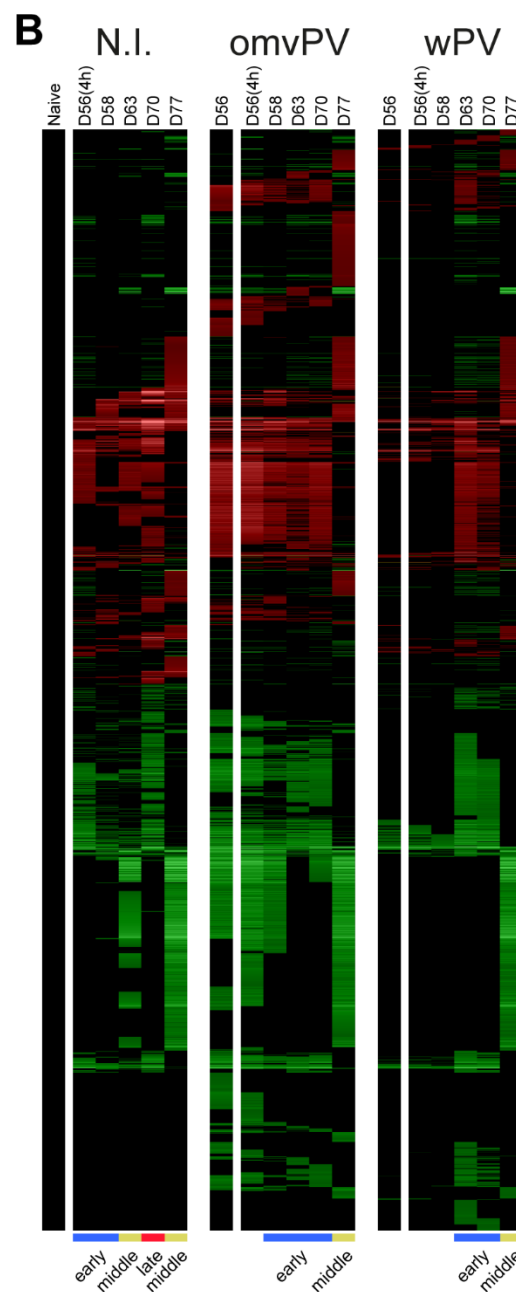
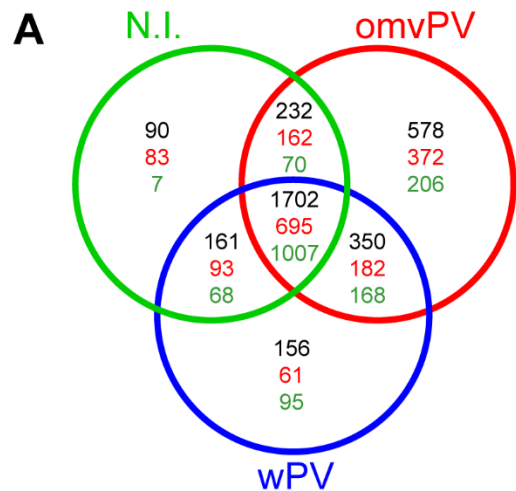
D



E



Supplementary Figure S4. Involvement of Gr1⁺ cells following omvPV and wPV booster immunization and after *B. pertussis* challenge. (A) Transcriptomic profiles from spleen tissue of omvPV-mice and wPV-mice were compared with BioGPS databases. The numbers of genes detected in the different BioGPS databases are listed. (B) Genes found following omvPV and/or wPV immunization that showed overlap with the Mac⁺Gr1⁺ granulocytes dataset. (C) Percentage of Gr1⁺ cells in the spleen following omvPV and wPV immunization as determined by using flow cytometry. Data presented as mean \pm SD (n = 4). * and ** = $p \leq 0.05$ and $p \leq 0.01$ for immunized mice vs. naive mice (day 0), ++ = $p \leq 0.01$ for omvPV-mice vs. wPV-mice. (D) Genes that matched with the Mac⁺Gr1⁺ granulocytes dataset in the transcriptomic profiles from spleen tissue of omvPV-, wPV-, and non-immunized mice following a *B. pertussis* challenge. (E) Percentage of Gr1⁺ cells in the spleen after challenge of immunized and non-immunized mice immunization as determined by using flow cytometry. Data are presented as mean \pm SD (n = 3). * and ** = $p \leq 0.05$ and $p \leq 0.01$ for challenged groups vs. day 56, # and ## = $p \leq 0.05$ and $p \leq 0.01$ for immunized groups vs. non-immunized group.



Supplementary Figure S5. Pulmonary transcriptomic profiles following *B. pertussis* challenge in omvPV-, wPV-, and non-immunized mice. (A) Fold changes in expression and significant gene expression were calculated compared to naive mice (FR ≥ 1.5 , p -value ≤ 0.001). In total, 3269 DEGs were found divided over the three groups in a Venn-diagram with total number of genes (black), upregulated genes (red), and downregulated genes (green). (B) All differentially upregulated (red) and downregulated (green) genes are portrayed as heatmap (mean of $n=3$ for immunized groups, $n=1$ (pool of 3 mice for non-immunized group)). Genes not surpassing a FR of 1.5 are shown as basal level (black). Gene clustering is based on up/downregulation, time of involvement, and presence in the different groups. Infection-induced responses were divided in phases according to the hierarchical clustering calculated in Supplementary Fig. S2C.